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Note

Separation of optical brighteners by liquid–solid chromatography. II*

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A great many different optical brighteners are available today, a major use of which is in household laundry detergent formulations. The choice of brightener for a given detergent is dependent on the washing conditions and the absorption characteristics of the textile fibers for which the detergent is to be used. However, of all the fluorescent brightening agents now being marketed, only seven compounds are currently used in the major detergents manufactured in this country[1]. Optical brighteners, as a class, have been of interest because of their apparent possible role as dermal sensitizers due to the deposition of the fluorescent agents on fabrics during manufacture and laundering and their subsequent migration from the treated fabrics to the skin[2]. Thus, the ability to separate and identify the compounds is important.

Optical brighteners are not comparable to bleaches, but are closely related to dyes, actually being dyes in the sense that they change the appearance of treated fabrics. To be suitable for use as an optical brightener, a compound must not absorb in the visible region of the spectrum, as this would result in a coloring of the fabric, but must absorb strongly in the near ultraviolet. In addition, the fluorescence must lie in the short-wavelength region of the visible spectrum, thus compensating for the blue light which has been absorbed by yellow fabric contaminants. Finally, the compound must be photochemically stable, be soluble or at least dispersable in aqueous media, and be adsorbed and held by the textile strongly enough to allow a significant build-up of the optical brightener on the fabric surface.

Although fluorescent whitening agents are present in detergents at only approximately 0.5% dry weight, they have become one of the more important detergent components in recent years. A typical detergent optical brightener blend consists of a bleach-stable cotton whitener, a brightener for nylon, and another for polyesters. Various optical brightener combinations are commonly used in detergent and laundry product formulations[3,4] to create an impression of superior brightness in fabrics after laundering.

In the course of a study of optical brighteners in this laboratory, it was decided to attempt an adaptation of Stensby's thin-layer chromatographic separation pro-

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cedure[5] to high-pressure liquid–solid chromatography[6] in the hope of achieving a more rapid separation of the compounds. Identification of the individual brightener molecules has become increasingly difficult owing to their growing numbers and structural variations. According to a recent Environmental Protection Agency study[2]: "reliable identification would depend on a detailed examination by chromatography, absorption spectroscopy, and a comparison of the infrared spectra to determine the parent structure and the substituents". While the substituents do not significantly affect emission, they alter the characteristic absorption into the textile fiber. Of the compounds listed in Fig. 1, numbers I, II, and IV are the most widely used detergent brighteners[7].

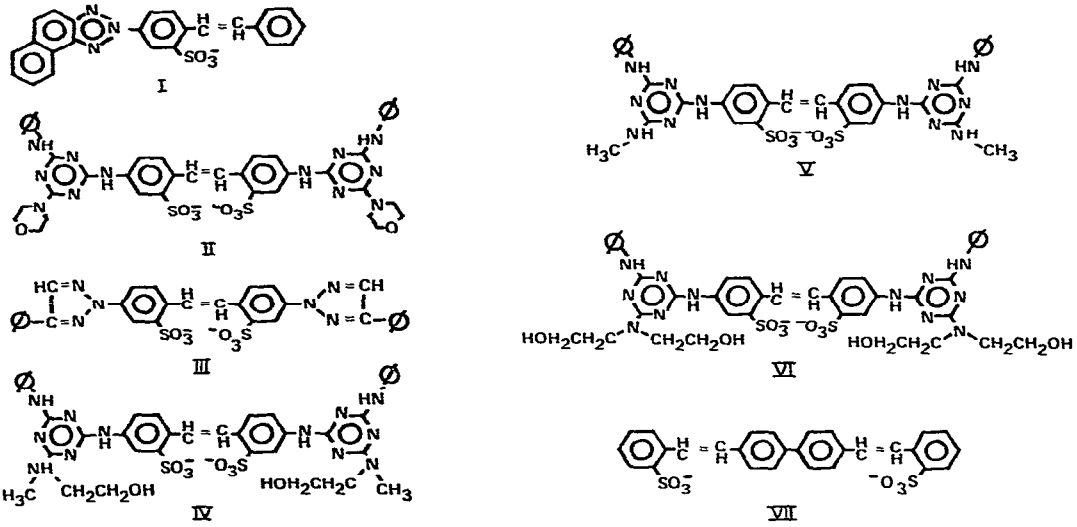


Fig. 1. Structures of fluorescent whitening agents. I = Naphthotriazolylstilbenesulfonate; II = bis-(anilinomorpholinotriazinylamino)stilbenedisulfonate; III = bis(phenyltriazolyl)stilbenedisulfonate; IV = bis(anilinohydroxyethylmethylamino-triazinylamino)stilbenedisulfonate; V = bis(anilinomethylaminotriazinylamino)stilbenedisulfonate; VI = bis(anilinodihydroxyethylaminotriazinylamino)stilbenedisulfonate; VII = bis(styrylsulfonate)biphenyl.

EXPERIMENTAL

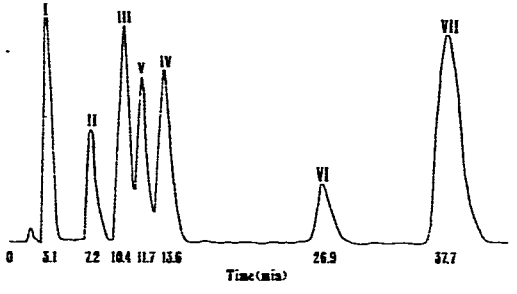
All solvents were purchased from Burdick and Jackson Labs, distilled-in-glass reagent grade for liquid chromatography and spectroscopy, with the exception of the ammonium hydroxide (ACS Reagent Code 1293), which was obtained from Allied Chemicals. The optical brightener samples were provided by Ciba-Geigy Corporation and the Verona Chemical Company.

Approximately 1 mg of each optical brightener sampled was dissolved in 50 ml of mobile phase (20 μg/ml, of which 10 μl or 0.2 μg was injected on to the column). The composition of the mobile phase used was benzene–*p*-dioxane–methanol–ammonium hydroxide (30%) (32:50:8:8). The chromatograph was a Spectra-Physics Model 3500 B liquid chromatograph fitted with a Valco (cv-6-HPA-N-60) 7000-p.s.i. manual injection valve; for the detection a Schoeffel Model 770 continuous wavelength UV/Vis detector was used.

RESULTS AND DISCUSSION

In the course of converting this method to a liquid chromatographic procedure, several approaches were tried. First, the above solvent system was used with a 50 cm × 2 mm I.D. Vydac silica column from Spectra-Physics (approximately 35-μm particle size) as were other solvents. Next, ion-exchange and reversed-phase chromatography were examined using Vydac ion exchangers and a Vydac reversed phase (35-μm particle size columns, 50 cm long) with the appropriate solvent mixtures (*e.g.*, an acetate buffered solvent system and gradient elution using 1% aqueous acetic acid *versus* acidified acetonitrile, respectively). Several different solvent systems including water, methanol, chloroform, hexane, etc., and variations of the final 32:50:8:8 mobile phase were used in this initial part of the experiment. None of the columns or solvent systems employed produced any significant separation of the brighteners.

A separation was first achieved using a MicroPak Si-5 silica gel column 25 cm long from Varian. The separation was achieved at room temperature with a flow-rate of 0.4 ml/min (Fig. 2), an absorbance range of 0.4 a.u.f.s., and a full-scale recorder deflection of 50 mV. The modification of the mobile phase from that used in the original thin-layer chromatographic study (40:50:20:10) produced a better separation of the optical brighteners III, IV, and V. In addition to the Varian silica column, separation of the seven optical brighteners was also achieved with a Whatman Partisil 10 silica micro-column, 25 cm × 4.6 mm I.D. As seen in Fig. 3, this column gave better resolution than was obtained with the Varian MicroPak Si-5 column. Fig. 4 is the chromatogram for the Whatman reversed-phase column (Partisil 10 ODS), which was run using the benzene–*p*-dioxane–methanol–ammonium hydroxide (32:50:8:8) mobile phase. Parameters were: sample injection volume, 10 μl; flow-rate, 0.12 ml/min; absorbance range, 0.04 a.u.f.s.; pressure, 370 p.s.i. As can be seen, only a partial separation was obtained; however, only one of the overlapping absorbances (brighteners IV + VII) represents a commonly used brightener (number IV), so that this column has possible application. A Varian 5-μm alumina column (MicroPak Al-5) 25 cm long was also investigated with marginal results. Resolution of the brighteners commonly used (I, II, and IV) from other brighteners was poor, with brightener IV appearing as a shoulder on the V, VII peak (Fig. 5). Parameters



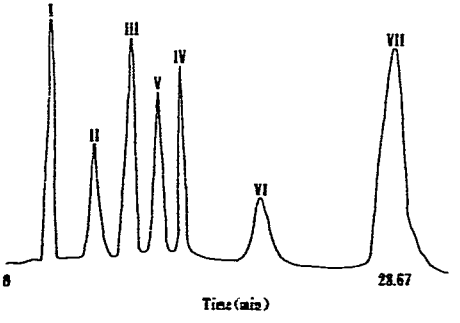
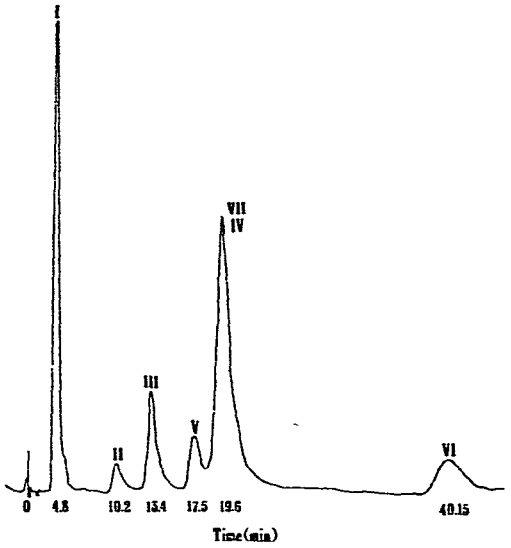


Fig. 2. Separation of optical brighteners on MicroPak Si-5 silica gel.

Fig. 3. Separation of optical brighteners on Partisil 10 silica gel.



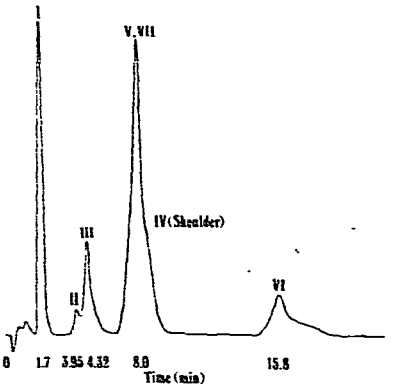


Fig. 4. Separation of optical brighteners on Partisil 10 ODS.

Fig. 5. Separation of optical brighteners on MicroPak Al-5.

for the alumina column were: absorbance range = 0.04 a.u.f.s., room temperature column and injection, pressure = 3920 p.s.i., and the flow-rate was 0.8 ml/min. Reduction of the flow-rate to 0.4 ml/min did not improve resolution. Column parameters are summarized in Table I for the 32:50:8:8 mobile phase data.

Detergent samples consisting of sodium dodecyl sulfate with an added amount of optical brightener (1–2 mg of brightener per 0.5–1.0 g of detergent) were extracted to determine whether the optical brighteners could be efficiently removed from such a detergent blend. Recovery studies showed that four extractions of 10 ml each with a solvent described by Stensby *et al.*[5], consisting of acetone–water–ammonium hydroxide (30%) in a ratio of 90:10:5 removed over 99% of the optical brightener. Recovery was estimated by the decreased ultraviolet absorption of the dissolved residue from the extraction. Brighteners from two commercially available detergents were successfully extracted by the same procedure. Quantitation and identification from commercial detergents has not been performed at this time.

Table II summarizes the formulae and the retention times for the seven optical brighteners studied on the two silica columns, the alumina column, and the reversed-phase column. All of these fluorescent whitening agents, which are bis(triazinyl) derivatives of 4,4′-diaminostilbene-2,2′-disulfonic acid, eluted with adequate resolution and in the same order on both of the silica columns as was observed for the thin-layer chromatographic technique. The commonly used brighteners were reasonably well separated on the reversed-phase column, but resolution with the alumina column was not acceptable.

TABLE I

COLUMN GEOMETRY AND PACKING MATERIALS

Column	*Manufacturer*	*Diameter (mm)*	*Length (cm)*	*Particle size (μm)*	*Packing*	*Pressure (p.s.i.)*	*Flow-rate (ml/min)*	*Temperature (°C)*	*Absorbance range (a.u.f.s.)*
MicroPak No. 188 Al-5	Varian	2.2	25	5	alumina	3920	0.80	25	0.04
MicroPak No. 249 Si-5	Varian	2.2	25	5	silica	2500	0.40	25	0.40
Partisil 10	Whatman	4.6	25	10	silica	3900	0.40	25	0.40
Partisil 10 ODS	Whatman	4.6	25	10	octadecyl-silane on silica	370	0.12	25	0.04

TABLE II

RETENTION TIMES (MIN) AND CAPACITY FACTORS (k')

Optical brightener	*Column*							
	MicroPak Si-5		*Partisil 10*		*Partisil 10 ODS*		*MicroPak Al-5*	
	t_R	k'	t_R	k'	t_R	k'	t_R	k'
I	3.1	0.72	2.7	0.48	4.8	1.40	1.7	0.67
II	7.2	3.00	3.2	0.79	10.2	4.10	3.9	2.95
III	10.4	4.78	8.4	3.66	13.4	5.70	4.3	3.32
IV	13.6	5.50	13.0	6.20	19.6	8.80	8.0	6.98
V	11.7	6.56	10.6	4.90	17.5	7.75	8.0	6.98
VI	26.9	13.94	15.0	7.30	40.2	19.75	15.8	14.75
VII	37.7	19.94	28.7	14.93	19.6	8.80	8.0	6.98

CONCLUSIONS

Separation of optical brighteners has been performed using micro-particle silica columns. Use of reversed-phase columns may have some application; however, the silica columns were found to provide higher resolution with generally shorter retention times than the reversed-phase column. Although the basicity of this mobile phase rapidly degrades silica columns, the ammonium hydroxide was found to be necessary for sample solubility.

Removal of the optical brighteners from detergent samples is feasible by solvent extraction with an acetone–water–ammonium hydroxide extractant.

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